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Diagnosis of Dengue NS 1 Antigen with a Finger Prick – Evaluation of New Rapid Diagnostic Test

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Authors' contributions

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ABSTRACT

Introduction: Dengue is one of the most common mosquito borne viral disease of humans and a major contributor to the health burden in the world.There are many reasons why early diagnosis of dengue is important. Hence there is need for Rapid Diagnostic Tests(RDTs) for early diagnosis of Dengue, that yields results within minutes and that can be performed in health centers with little infrastructure or trained personnel, preferably without electricity.
Aims: To identify differences in sensitivity, specificity, and likelihood ratios between the diagnostic assays. To determine the positive and negative predictive value of the Rapid NS1-FP(Finger Prick) test and To correlate platelet counts with expression of NS 1 antigen.
Study Design: Prospective case control study.
Place and Duration of Study: Blood samples were collected from 200 patients clinically suspected of dengue during JUNE – 2017 to AUGUST – 2017 form PADMASHREE DIAGNOSTIC CENTER, BANGALORE.
Methodology: Dengue suspects were evaluated according to WHO criteria for probable dengue infection. Blood samples were collected from 200 patients clinically suspected of dengue were tested for NS 1 antigen using Serum card method and Finger prick method and results were compared with ELISA method ,later the qualitative results of NS 1 antigen were correlated with platelet counts.
Results: Total number of male cases were 113 (56.5%) and female cases were 87 (43.5%). The

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Sensitivity and specificity rates were on par with serum card method. The performance characteristics of Whole blood finger prick method was as follows.1.Sensivity – 90.9% 2.Specificity – 100%

Conclusion: Further studies are required to assess the potential impact of implementing early laboratory diagnosis of Dengue in terms of prognosis and cost effectiveness.

Keywords: Dengue diagnosis; NS 1; Platelet count; whole blood method; finger prick method.

1. INTRODUCTION

Dengue is one of the most common mosquito borne viral disease of humans and a major contributor to the health burden in the world.The overall estimate of the prevalence of laboratory confirmed dengue infection among clinically suspected patients was 38.3% [1]. A recent review has reported that India alone contributes to 34% (about 33 million infections) of the total global threat of dengue leading to hyper-endemicity, prevailing mostly in urban areas and now spreading to rural areas [2].

Dengue fever (DF) is commonly benign, is defined as acute febrile illness with two or more manifestation among headache, retro-orbital pain, myalgia, arthralgia. Haemorrhagic manifestation i.e., skin haemorrhage with tourniquet test, and petechiae are not uncommon. Few cases of epistaxis, gingival bleeding, gastrointestinal bleeding, hematuria and hyper-menorrhagia have been reported [3]. A number of atypical manifestations are also reported which include encephalitis, encephalopathy, myocarditis, hepatitis and cholecystitis. Dengue is an international public health problem, even travelers who travel to the dengue affected areas are expected the possibility of infection [4].

Dengue virus is a single stranded enveloped RNA virus belonging to the genus flavivirus in the family Flaviviridae and transmitted through the bites of Aedesaegypti and Aedesalbopictus mosqitoes.[5] There are five serotypes of the virus distinguished on the basis of antigenicity; first four are referred to as DENV-1, DENV-2, DENV-3 and DENV-4, and the fifth type was announced in 2013 [5]. Dengue NS 1 antigen is a sensitive and most useful specific marker for early diagnosis of dengue virus infection and improves the diagnostic accuracy in combination with antibody test [6]. The Diagnostic methods available are virus isolation, genomic RNA by RTPCR and IgG, IgM detection by ELISA.

There are many reasons why early diagnosis of dengue is important. Firstly it can assist in patient management and secondly it prevents unnecessary and expensive antibiotic usage. Thirdly, prompt diagnosis of the index cases and lastly provides important data on the epidemiology and health burden of dengue [7]. Apart from dengue specific parameters, thrombocytopenia and hemoconcentration are constant findings in DHF. A drop in platelet count below 1,00,000 per mm3 is usually found between the third and eight day of illness [8].

Hence there is need for Rapid Diagnostic Tests(RDTs) for early diagnosis of Dengue, that yields results within minutes and that can be performed in health centers with little infrastructure or trained personnel, preferably without electricity [9].

The main aim of this study is to evaluate one such RDTs using whole blood by finger prick method in comparison with ELISA method and Serum card method that are being used currently for NS 1 detection and correlate the platelet count with various dengue specific serological markers by immunochromatography based test in a healthcare setup.

2. MATERIALS AND METHODS

Dengue suspects were defined as patients presenting with acute febrile illness, rashes, bleeding tendencies, leucopenia or thrombocytopenia were evaluated according to WHO criteria for probable dengue infection [10]. A total of 200Blood samples were collected from patients clinically suspected of dengue during JUNE – 2017 to AUGUST – 2017 form PADMASHREE DIAGNOSTIC CENTER, BANGALORE.

2.1 Inclusion Criteria

Patients presenting with acute febrile illness, rashes, bleeding tendencies, leucopenia or thrombocytopenia were considered for probable dengue infection.

2.2 Exclusion Criteria

Healthy individuals, typical symptoms of other diseases, hemolyzed samples, icteric samples, lipemic samples.

2.3 Specimen Handling and Analysis

The blood specimens received in the laboratory was centrifuged (1800 x g /15mins) to separate the cellular components and the cell free serum processed for the analysis of routine biochemical parameters sought by the treating clinicians. Remaining specimens were aliquoted, labeled and stored at – 20ºC till further analysis. Aliquots of specimens, once thawed were used for the analysis on the same day and not be subjected to repeat freezing and thawing to avoid any pre-analytical errors.

All the 200 sample were tested for NS 1 antigen using conventional Serum card method and ELISA method .Consent was taken form the patiets and the finger prick method was tested at the time of phlebotomy. Dengue Ns1 antigen FP is a rapid solid phase immune- chromatographic test for the qualitative detection of dengue NS1 antigen in human whole blood/ serum/ plasma. This test is for invitro diagnostic use only and is intended as an aid in the earlier diagnosis of dengue infection.Platelet count was documented using automated and manual method.

The results were tabulated and the sensitivity, specificity patterns were determined and compared using the Unpaired t-test and P value calculated using the SPSS soft ware version 21.0.

3. RESULTS AND DISCUSSION

Among the 200 sample tested , total number of male cases were 113 (56.5%) and female cases were 87 (43.5%). The mean age of occurrence in males was 16.65±16.20 years and in females it was 19.11±15.06 years.

Table 2 shows the measure of platelet counts over the positive and negative results according to the tests procedures. It is seen that the average platelet count in NS1 positive cases was 1.94 lakhs, were as in NS1 negative cases it was 2.52 lakhs.

There was significant variation of platelet counts among the positive and negative subjects , with P value <0.0001 among all the tests types.Calculations were done on the basis of the following formulas.

Sensitivity=[a/(a+c)]×100

Specificity=[d/(b+d)]×100

Positive predictive value(PPV)=[a/(a+b)]×100

Negative predictive value(NPV)=[d/(c+d)]×100.

Table 1. Compares the outcomes of diagnostic efficacy of serum card method and Whole blood method compared to reference test(ELISA)

Sl. No	Measures	Serum card method over reference test	Whole blood method over reference test
1	Sensitivity	92.4%	90.9%
2	Specificity	100%	100%
3	Positive predictive value (PPV)	100%	100%
4	Negative predictive value	96.4%	95.7%
5	LR positive	0	0
6	LR Negative	0.076	0.091

Table 2. Measurement of platelet counts over the positive and negative results

Sl no	Diagnostic tests	Positive	Negative	Unpaired t-test value	p-value
		Mean±SD	Mean ±SD		
1	Reference test(ELISA)	1.93±0.92	2.55±0.94	4.36	P<0.001
2	Serum card method	1.95±0.94	2.51±0.98	3.76	P<0.001
3	Whole blood method	1.96±0.95	2.52±0.0.95	3.75	P<0.001

3.1 Discussion

In the present study we compared simultaneously the performance of ELISA method, serum card method and Whole blood finger prick method for detection of NS 1 antigen for early diagnosis of dengue. The total sample size was 200 random samples with non-specific clinical symptoms of Dengue fever.

Early detection of illness, careful monitoring and appropriate fluid therapy alone have decreased mortality to 1%. If shock is identified when pulse pressure starts to drop and intravenous fluids are administered, the outcome will be excellent. Recovery is fast and most patients recover in 24-48 hours without any sequela. The outcome may not be so good if the patient develops cold extremities. Most deaths from DHF/DSS are caused by prolonged shock, massive bleeding, fluid overload and acute liver failure with encephalopathy [14]. Hence in a populated country like India, Dengue diagnosis using the whole blood finger prick method would prove to be a handy tool in early diagnosis.

Thrombocytopenia is used as one of the criteria by WHO guidelines as a strong indicator of clinical severity of Dengue infection. The Platelet count is a very important variable and is a affordable laboratory test that can be estimated in a primary health care set up. The platelet counts were compared between dengue positive and negative patients (Table 5).

The Platelet counts in Dengue positive and negative cases showed significant difference of almost 50,000 to 60,000 cells/cumm. But the values in comparison with other study did not correlate as the positive cases in study conducted by Ramandeep et al15 showed average platelet count of 34,000 cells/cumm. This difference would be due to the geographic variation and individuality of the cases.

The striking increase in the urban and rural load of dengue , has gained increased consiousness in developing rapid diagnostics for dengue infections. The ELISA test has greater sensitivity ans specificity in detecting dengue antibodies than the rapid tests, but the rapid tests are fast,

Table 3. Compares the sensitivity and specificity pattern of various rapid diagnostic tests

Sl no	Card used	Sensitivity %	Specificity %
1	SD Bioline Osorio et al. [11]	51(95 % CI)	96.7(95 % CI)
2	Biorad Om Prakash et al. [12]	79.1(95 % CI)	100(95 % CI)
3	Panbio Om Prakash et al. [12]	71.9(95 % CI)	95(95 % CI)
4	J Mitra Whole blood method (present study)	90.9(95 % CI)	100(95 % CI)

Table 4. Sensitivity and specificity pattern of various rapid diagnostic tests

Sl no	Card used	Sensitivity %	Specificity %	PPV %	NPV %
1	Platelia NS 1 antigen Shenoy et al. [13]	83.6	98.7	98.3	86.3
2	NS 1 antigen strip kit Shenoy et al. [13]	89.6	99	99.5	91.1
3	J Mitra serum card method	92.4	100	100	96.4
4	J Mitra whole blood method	90.9	100	100	95.7

Table 5. Comparison of platelet counts in dengue positive and negative cases

Sl no	Study name	Platelet counts cells/cumm in positive cases	Platelet counts cells/cumm in negative cases
1	Ramandeep et al15	0.34	2.20
2	Our study	1.94	2.52

convenient, technician independent, with the results available in a quick time. This will be very helpful in starting treatment and minimizing the morbidity and mortality of dengue infection. Contemplating the increasing burden of dengue in our country, introduction of rapid diagnostic tests for speedy and accurate diagnosis would be the need of the hour.

4. CONCLUSION

The present study shows that Whole blood finger prick method can be used as a screening test for dengue in early diagnosis of NS 1 antigen. The Sensitivity and specificity rates were are on par with serum card method.

The performance characteristics of Whole blood finger prick method was as follows.1.Sensivity – 90.9 % 2.Specificity – 100 % 3.Positive predictive value – 100 % .Hence this method serves as a valuable investigation for early detection of NS 1 antigen. The Platelet values too showed significant variation in NS1 positive and negative cases. This method was highly reproducible. Clinicians must be aware that a negative test does not rule out dengue infection. To take evidence based – decisions about the effectiveness of this test in clinical settings, it is recommended to assess its performance in consecutive subjects with potential dengue infection under routine conditions at health centers with different levels of complexity. Further studies are required to assess the potential impact of implementing early laboratory diagnosis of Dengue in terms of prognosis and cost effectiveness.

CONSENT AND ETHICAL APPROVAL

Ethical clearance was obtained from the institutional review board and the informed consent taken from the patient.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K, Anandaselvasankar A, et al. Dengue infection in India: A systematic review and meta-analysis. PLoS Negl Trop Dis. 2018;12(7):e0006618. Available:https://doi.org/10.1371/journal.pntd.0006618
2. Asim Sarfraz a, Nitesh Jaiswal a, Pradeep Das b. Impediments of reporting dengue cases in India. Journal of Infection and Public Health. 2017;10:494–498.
3. Tewari KN, Tuli NR, Devgun SC. Clinical profile of dengue fever and use of platelets in four tertiary level hospitals of Delhi in year 2009.Journal Indian Academy Of Clinical Medicine. 2013;14(1):8-12.
4. Pradesi Naidu A, Venkateswarlu P, Umadevi S, Sailaja M, Praveena B, Renuka A, et al. Detection of dengue NS1 by a CComparative analysis of panbio elisa and rapid diagnostic test. Scholars Journal of Applied Medical Sciences. 2017;5(3b):816-820.
5. Day-Yu Chao , Yi-Jung Liu, Wen-Fan Shen, Wu-Chun Tu, Jedhan Ucat Galua, Han-Chung Wu. Comparison of E and NS1 antigens capture ELISA to detect dengue viral antigens from mosquitoes. Journal of Vector Borne Disease. 2015;52:134-141.
6. Amol Hartalkar, Sheetal Hartalkar, Makarand Wani, Arvind Yadav. Dengue NS1 antigen – for early detection of dengue virus infection . WIM JOURNAL. 2015;2(1):11-15.
7. VianneyTricou, Hang TT Vu, Nhu VN Quynh, Chau VV Nguyen, Hien T Tran, Jeremy Farrar, et al, Comparision of two dengue NS1 rapid tests for sensitivity , specificity and relationship to viraemia and antibody responses, BioMed Central infectious diseases. 2010;10(142):1-8.
8. Santosh Shivajirao Tathe, Chincholkarvv, Kulkarni DM, Nilekar SL, et al. A study of NS1 antigen and platelet count for early diagnosis of dengue infection, International Journal of Current Microbiology and Applied Sciences. 2013;2(12):40-44.
9. Chappuis F, Alirol E, Acremont Vd, Bottieau E, Yansouni CP. Rapid diagnostic tests for non-malarial febrile illiness in the tropics, Clinical microbiology and infection, European society of clinical microbiology and infectious diseases. 2013;19:422-431.
10. Maria G Guznan , Suott B Halstead, Harvey Artsob, Philippe buchy, Jeremy Farrar, Dunae J Gubler, et al, Dengue : A continuing global threat evaluating diagnostics. Nature reviews Microbiology. 2010;S7-S15.

11. Lyda Osorio, Meleny Ramirez, Anilzabonelo, Luis A Villar, Beatriz Parra. Comparision of the diagnostic accuracy of commercial NS1-based diagnostic tests for early dengue infection. Virology Journal. 2010;7(361):1-10.
12. Om Prakash, Rafidah Hanim Shueb. Diagnosis of dengue infection using conventional and biosensor based techniques. 2015;7(10):5410-5427.
13. Bhaskar Shenoy, Anupama Menon, Suvarna Biradar. Diagnostic utility of dengue NS1 antigen. pediatric infectious disease. 2014;6:110-113.
14. Fever: Management issues in an intensive care unit. Journal de pediatria. 2007 ;83(2):S22-S35.

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